human genome in order to create high density SNP maps demonstrating that the techniques described in the specification are easily scalable and can be used for mapping a genome. Exemplary large maps that have been completed include a region of chromosome 12 approaching 10MB and an approximately 5MB region on chromosome 13q31-33.

Moreover, further downstream, for several of these regions genotyping and association analysis to detect disease loci have already been successfully carried out using the maps. For example, in the latter (chr. 13q31-q33) region, the present method was used to generate a high density SNP map having a heterozygosity rate of at least 0.18. The frequency of SNPs was determined, confirming said heterozygosity rate, and genotyping and association analysis with schizophrenia was carried out using 132 SNPs at an average density of 35kb. Subsequently, Applicants succeeded in identifying statistically significant associations of SNP alleles with disease and identified candidate diseases genes located in said chromosomal region.

II. Specification

In the Office Action mailed January 10, 2001, the Examiner requested that the trademark TWEEN 20 be capitalized and the generic terminology be provided. Applicants have amended the specification in accordance with the Examiner's request.

In the telephonic interview of March 29, 2001, the Examiner requested that the current status of cited applications be updated in the specification. In accordance with the Examiner's request, Applicants have amended the specification to reflect the fact that U.S. Patent Application Serial Number 09/058,746 is now U.S. Patent Number 6,022,716 and U.S. Patent Application Serial Number 08/996,306 is now U.S. Patent Number 5,945,522.

III. Rejection of Claims 86-105 Under 35 U.S.C. §112, first paragraph

Claims 86-105 were rejected under the first paragraph of 35 U.S.C. §112 on the assertion that the terminology "biallelic markers" required that the markers be located within a gene. As acknowledged by the Examiner in the interview summary mailed March 29, 2001, the replacement of the terminology "biallelic markers" with the terminology "single nucleotide polymorphisms" in the claims as amended above renders these concerns moot.

Furthermore, as requested by the Examiner, Applicants have amended the claims to specify that the nucleic acid library is a human nucleic acid library.

IV. Rejection of Claims 86-105 Under 35 U.S.C. §112, Second Paragraph

Claims 86-105 were rejected under the second paragraph of 35 U.S.C. §112 on the assertion that the claims were unclear and missing essential steps because it was unclear how it was determined that the sequence differences were located within genes. As discussed above and acknowledged by the Examiner in the interview summary mailed March 29, 2001, these concerns are rendered moot in view of the claims as amended above.

Claim 87 was rejected under the second paragraph of 35 U.S.C. §112 on the assertion that the terminology "minimally" was unclear. Applicants maintain that the terminology "minimally overlappling" is a standard term recognized by those skilled in the art and that Claim 87 is patentable as written. However, solely for the purposes of expediting the prosecution of the present Application, Claim 87 has been cancelled. Applicants maintain full rights to pursue the subject matter of Claim 87 in related applications without limitation.

Claims 102-105 were rejected under the second paragraph of 35 U.S.C. §112 on the assertion that the terminology "proximity" was indefinite. This terminology has been deleted in the claims as amended above. Applicants note that the terminology "in linkage disequilibrium with one another" which has been added to Claim 102 is supported throughout the specification, including at page 2, lines 29 through 32, page 33, line 14 through page 38, line 31, and page 42, line 36 through page 43, line 2.

## CONCLUSION

In view of the foregoing, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the rejections is respectfully requested. Should the Examiner have any questions regarding this matter he is invited to telephone the undersigned so that the questions may be resolved.

The specific changes to the specification and the amended claims are shown on a separate set of pages attached hereto and entitled **VERSION WITH MARKINGS TO SHOW**

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**CHANGES MADE**, which follows the signature page of this Amendment. On this set of pages, the insertions are double underlined while the ~~deletions are stricken through~~.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

**The paragraph beginning on page 17, line 5 has been amended as follows:**

Alternatively, the genomic DNA may be inserted into BAC vectors which possess both a high copy number origin of replication, which facilitates the isolation of the vector DNA, and a low copy number origin of replication. Cloning of a genomic DNA insert into the high copy number origin of replication inactivates the origin such that clones containing a genomic insert replicate at low copy number. The low copy number of clones having a genomic insert therein permits the inserts to be stably maintained. In addition, selection procedures may be designed which enable low copy number plasmids (i.e. vectors having genomic inserts therein) to be selected. Such vectors and selection procedures are described in the U.S. Patent Application entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No. 09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

**The paragraph beginning on page 20, line 20 has been amended as follows:**

Alternatively, BAC subcloning may be performed using vectors which possess both a high copy number origin of replication, which facilitates the isolation of the vector DNA, and a low copy number origin of replication. Cloning of a genomic DNA fragment into the high copy number origin of replication inactivates the origin such that clones containing a genomic insert replicate at low copy number. The low copy number of clones having a genomic insert therein permits the inserts to be stably maintained. In addition, selection procedures may be designed which enable low copy number plasmids (i.e. vectors having genomic inserts therein) to be selected. In a preferred embodiment, BAC subcloning will be performed in vectors having the above described features and moreover enabling high throughput sequencing of long fragments of genomic DNA. Such high throughput high quality sequencing may be obtained after generating successive deletions within the subcloned fragments to be sequenced, using transposition-based or enzymatic systems. Such vectors are described in the U.S. Patent Application entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No.

09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

**The paragraph beginning on page 21, line 19 has been amended as follows:**

As a preferred alternative to sequencing the ends of an adequate number of BAC subclones, the above mentioned high throughput deletion-based sequencing vectors, which allow the generation of a high quality sequence information covering fragments of ca. 6kb, may be used. Having sequence fragments longer than 2.5 or 3kb enhances the chances of identifying biallelic markers therein. Methods of constructing and sequencing a nested set of deletions are disclosed in the U.S. Patent Application entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No. 09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

**The paragraph beginning on page 49, line 27 has been amended as follows:**

In practice this microsequencing analysis is performed as follows. 20 µl of the microsequencing reaction is added to 80 µl of capture buffer (SSC 2X, 2.5% PEG 8000, 0.25 M Tris pH7.5, 1.8% BSA, 0.05% ~~Tween~~ TWEEN® 20 (polyoxyethylene (20) sorbitan monopalmitate)) and incubated for 20 minutes on a microtiter plate coated with streptavidin (Boehringer). The plate is rinsed once with washing buffer (0.1 M Tris pH 7.5, 0.1 M NaCl, 0.1% Tween 20). 100 µl of anti-fluorescein antibody conjugated with phosphatase alkaline, diluted 1/5000 in washing buffer containing 1.8% BSA is added to the microtiter plate. The antibody is incubated on the microtiter plate for 20 minutes. After washing the microtiter plate four times, 100 µl of 4-methylumbelliferyl phosphate (Sigma) diluted to 0.4 mg/ml in 0.1 M diethanolamine pH 9.6, 10mM $MgCl_2$ are added. The detection of the microsequencing reaction is carried out on a fluorimeter (Dynatech) after 20 minutes of incubation.

**The paragraph beginning on page 52, line 8 has been amended as follows:**

Examples 15-23 illustrate the application of the above methods using biallelic markers to identify a gene associated with a complex disease, prostate cancer, within a ca. 450 kb candidate region. ~~Additional~~ Additional details of the identification of the gene associated with prostate

cancer are provided in the U.S. Patent Application entitled "Prostate Cancer Gene" (GENSET.018A, Serial No. 08/996,306, now U.S. Patent Number 5,945,522), the disclosure of which is incorporated herein by reference.

IN THE CLAIMS:

86. (Amended) A method of obtaining a plurality of ~~biallelic markers~~ single nucleotide polymorphisms comprising the steps of:

(a) obtaining a human nucleic acid library comprising a plurality of genomic DNA fragments ~~comprising~~ containing the full human genome or a portion ~~thereof~~ of the human genome comprising at least 100kb;

(b) determining the order of said plurality of genomic DNA fragments in the genome;

(c) ~~determining the sequence of~~ sequencing selected regions of said plurality of genomic DNA fragments; and

(d) identifying nucleotides in said ~~plurality of genomic DNA fragments~~ selected regions which vary between individuals, thereby defining a set of ~~biallelic markers~~ single nucleotide polymorphisms; wherein said plurality of single nucleotide polymorphisms comprises single nucleotide polymorphisms having a heterozygosity rate of at least about 0.18 and having a mean inter-marker spacing of less than 50kb.

87. Cancelled.

88. (Amended) The method of Claim 86, further comprising identifying one ~~biallelic marker~~ single nucleotide polymorphism per genomic DNA fragment.

89. (Amended) The method of Claim 86, further comprising identifying two or more ~~biallelic markers~~ single nucleotide polymorphisms per genomic DNA fragment.

90. Cancelled.

91. Cancelled.

92. (Amended) The method of Claim 86, further comprising selecting ~~biallelic markers~~ single nucleotide polymorphisms having a heterozygosity rate of ~~at least~~ about 0.32.

93. (Amended) The method of Claim 86, further comprising selecting ~~biallelic markers~~ single nucleotide polymorphisms having a heterozygosity rate of ~~at least~~ about 0.42.

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94. (Amended) The method of Claim 86, wherein said identifying step comprises identifying at least ~~about~~ 20,000 ~~biallelic markers~~ single nucleotide polymorphisms.

97. (Amended) The method of Claim 86, further comprising determining the position of said ~~biallelic markers~~ single nucleotide polymorphisms along the genome or a portion ~~thereof~~ of the genome.

98. (Amended) The method of Claim 86, further comprising obtaining pluralities of ~~biallelic markers~~ single nucleotide polymorphisms such that each ~~marker~~ single nucleotide polymorphism is in linkage disequilibrium with at least one of said identified ~~markers~~ single nucleotide polymorphism.

102. (Amended) The method of Claim 86, further comprising the step of identifying one or more groups of ~~biallelic markers~~ single nucleotide polymorphisms which are in ~~proximity to one another in the genome~~ linkage disequilibrium with one another.

103. (Amended) The method of Claim 86, further comprising the step of identifying one or more groups of ~~biallelic markers~~ single nucleotide polymorphisms ~~which are in proximity to one another in the genome,~~ wherein the ~~biallelic markers~~ single nucleotide polymorphisms in each of these groups are located within a genomic region spanning from 1 to 5kb.

104. (Amended) The method of Claim 86, further comprising the step of identifying one or more groups of ~~biallelic markers~~ single nucleotide polymorphisms ~~which are in proximity to one another in the genome,~~ wherein the ~~biallelic markers~~ single nucleotide polymorphisms in each of these groups are located within a genomic region spanning from 50 to 150kb.

105. (Amended) The method of Claim 86, further comprising the step of identifying one or more groups of ~~biallelic markers~~ single nucleotide polymorphisms ~~which are in proximity to one another in the genome,~~ wherein the ~~biallelic markers~~ single nucleotide polymorphisms in each of these groups are located within a genomic region spanning more than 1Mb.